DNA Technical Information

Adapted by Lori Yang from www.biosyn.com/t_dna.htm

I. Calculations

A. Using Absorbance at 260 nm to Measure DNA Concentration

A useful estimation of DNA concentration relates to the amount of oligonucleotide which, when dissolved in 1 mL of water, results in an absorbance of 1 when measured at 260 nm in a 1 cm path length cuvette. This is often simply called the A_{260} of a sample. The actual concentration can range from 39 µg/mL (for a homopolymer of C) to 20 µg/mL (for a homopolymer of A). For most practical experiments, an A_{260} of 1.0 represents approximately 33 mg of oligo with an equal mixture of the four bases.

A_{260} conversion factors

A_{260} = 1.0 ➔ 33 µg/mL ssDNA
➔ 40 µg/mL ssRNA
➔ 50 µg/mL dsDNA

Molecular Weight of an Oligonucleotide

MW_{oligo} = ((A x 312.2)+(G x 328.2)+(C x 288.2)+(T x 303.2)-61)
where A,C,G,T represent the number of A's, C's, G's and T's in an oligo.

Molar conversions

1 µg of 1,000 bp DNA = 1.52 pmol (3.03 pmoles of ends)
1 µg of pBR322 DNA = 0.36 pmol DNA
1pmol of 1,000 bp DNA = 0.66 µg
1pmol of pBR322 DNA = 2.78 µg

Melting Temperature (Nucleic Acid Hybridization)

Up to 25 bp:

T_m = 4ºC (G+C) + 2ºC (A+T)

More than 25 bp:

T_m = 81.5ºC + 16.6 log M + 0.41 %(G+C) – 500 / n – 0.61 (% formamide)

M = $[Na_+]$ in moles/liter ; n = length of shortest chain in duplex

B. Other Information

Resuspension Buffers

1. Sterile Water (dd H_2O)
2. TE Buffer (10mM Tris-HCI, 1mM EDTA) pH 7.5

DNA Storage Conditions and Stability

Lyophilized (-20˚C) = 6 months to several years
Lyophilized (25˚C) = 2 months to 1 year
Dissolved (-20˚C) = 1 month to 6 months
Dissolved (25˚C) = 1 week to 3 months

DNA Conformations

Helix type	Direction of rotation	Residues per turn	Rotation per residue	Helix rise per residue	Helix pitch
A	Right	11	33º	2.55 Å	28 Å
B	Right	10	36º	3.4 Å	34 Å
Z	Left	12	–30º	3.7 Å	45 Å

PAGE Purification Information

Dye migration on a denaturing gel (7 M UREA), 1x TBE

% Gel	*Separation range (bases)*	Bromophenol Blue	Xylene Cyanol
5.0	100-200	35	140
8.0	80-150	20	75
12.0	40-100	12	53
15.0	12-80	10	44
20.0	8-60	8	24

References

1. Suggs, S., et. al. Proc. Natl. Acad. Sci 78:6613('81)
2. George H. Keller, Mark M. Manak, DNA probes; p 15; M Stockton Press, '89.